IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of: T. Kosoglou et al.	Examiner: San-Ming R. Hui
Serial No.: 10/056,680	Group Art Unit: 1617
Filed: January 25, 2002	Atty. Docket No.: CV01492K
For: Combinations of Sterol Absorption Inhibitor(s) with Blood Modifiers for Treating Vascular Indications	

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DECLARATION OF MADHU CHINTALA, Ph.D.

I, Madhu Chintala, declare and state that:

1. I obtained a Bachelor of Science degree in Zoology from the University of Madras, India in 1984.

2. I obtained a Master of Science degree in Ocean Life Sciences from the University of Madras, India in 1985.

3. I obtained a Doctorate Degree in Pharmacology from the University of Houston in 1991.

4. I am employed by Schering-Plough Research Institute ("Schering") as an Associate Director in the field of Cardiovascular and Metabolic Disease and have been employed in this capacity since 2003 and was previously employed by Schering as a scientist since 1991.

5. My duties at Schering have included pharmaceutical drug discovery and basic research in the areas of atherothrombosis, heart failure, lipid disorders and metabolic diseases.

6. A study was conducted under my supervision to determine if a reduction in plasma cholesterol levels by ezetimibe (EZ) would enhance the ability of aspirin (ASA) to act as a platelet aggregation inhibitor. Rats were fed a 1% cholesterol + 0.5% cholate diet (HC) alone or containing ezetimibe (0.0036%, 3 mg/kg/day) for 7 days. On day 7 they were treated with aspirin at 100 mg/kg or vehicle, and platelet aggregation determined. Mean plasma cholesterol levels were reduced from 344 ± 22 mg/dl to 60 ± 4 mg/dl by ezetimibe treatment. Platelet aggregation by adenosine diphosphate (ADP) and collagen was not altered, as expected, among the groups. Arachidonic acid (AA) induced platelet aggregation at 0.3 mM was increased by the hypercholesterolemic diet compared to normal chow fed rats (Table), indicating an increased sensitivity to aggregate with hypercholesterolemia. AA induced aggregation was not reduced in the aspirin alone treated hypercholesterolemic animals. AA induced aggregation was significantly reduced in the aspirin + ezetimibe treated rats compared to the aspirin alone treated hypercholesterolemic rats (Table).

Table: Platelet Aggregation

Agonist	Regular Chow	High Cholesterol (HC) diet	HC + EZ	HC + ASA (100 mpk)	HC + EZ + ASA (100 mpk)
AA (0.3 mM)	7 ± 3	14 ± 2	13 ± 2	12 ± 2	7 ± 2
AA (1 mM)	16 ± 2	17 ± 3	16 ± 3	14 ± 2	5 ± 2
ADP (10 μM)	24 ±1	21 ± 2	21 ± 3	25 ± 2	31 ± 1
Collagen (3 μg/ml)	25 ± 1	24 ± 3	27 ± 3	30 ± 2	32 ± 1

Aggregation in whole blood (ohms)

N=6 per group, Mean ± SEM

Rationale for dose selection of Aspirin and Ezetimibe for the above study in rats

7. Aspirin is an antiplatelet agent which is widely used to prevent atherothrombosis in the treatment of cardiovascular disorders including stroke. Aspirin exerts its beneficial effects by inhibiting platelet aggregation and thrombus formation also commonly referred to as blood clots. Ex-vivo platelet aggregation (a measure of platelet function) has widely been used as a surrogate for antithrombotic activity and for determining the therapeutic doses of aspirin in humans and in animals. The dose of aspirin used clinically to treat patients varies depending on the indication/disease conditions. A standard dose of 100 mg/day was shown sufficient to inhibit platelet aggregation in 90% of patients in primary and secondary prevention of cardiovascular diseases.[1] Doses of 300 and 600 mg/day were required in stroke patients with single or recurring events.[2] Higher doses of 500-1000 mg were found effective in the treatment of fever and other symptoms of upper respiratory tract infection in adults[3], in the treatment of episodic tension-type

[1] Syrebe et al., *Individual Dosing of ASA Prophylaxis by Controlling Platelet Aggregation*, Clin. Appl. Thromb. Hemost. Jul; 7(3): 209-13, 2001.
[2] Chamorro et al., *Ex-Vivo Responses to Aspirin Differs in Stroke Patients with Single or Recurrent Events: A Pilot Study*, J. Neurol. Sci. Dec 15; 171(2); 110-4, 1999.
[3] Bachert et al., *Aspirin Compared with Acetaminophen in the Treatment of Fever and Other Symptoms of Upper Respiratory Tract Infection in Adults: A Multicenter, Randomized, Double-Blind, Double-Dummy, Placebo-Controlled, Parallel-Group, Single-Dose, 6 Hour Dose-Ranging Study*, Clin. Ther. Jul; 27 (7): 993-1003, 2005.

headache[4], and to prevent platelet activation in patients before and after percutaneous coronary interventions[5]. Thus the dose of aspirin varies in humans depending on the clinical indication and it is reasonable to assume that the therapeutic range is from 1-1000 mg.

8. In the above rat studies, we used aspirin at 100 mg/kg, orally. In our experience this dose of aspirin is necessary to inhibit platelet aggregation in rats 1-2 hrs after oral dosing. Studies in the literature have employed different doses of aspirin in rats and the dose varies upon the route of administration and the type of injury/thrombosis model used. In a rat model of laser-induced thrombosis, administration of aspirin at 100 mg/kg prevented thrombus formation.[6] In a similar laser-injury model, Vesvres et al, 1993, have shown that doses of 50, 100 and 200 mg/kg, administered intramuscularly prevented thrombus formation in a dose dependent manner.[7] Killackey et al, 1984, have reported that they required 200 mg/kg of aspirin to prevent carotid artery thrombosis in a rat model and that the 100 mg/kg dose was insufficient.[8] In contrast, several reports have shown that doses of aspirin ranging from 1-50 mg/kg did not significantly inhibit thrombus formation in rats[9,10] or they had modest effects.[11,12] Thus, the therapeutic dose of aspirin in rats is around 100 mg/kg. Hence the dose used in our studies in the therapeutic range for prevention of thrombosis in rats is consistent with reports in the literature. While on an mg/kg basis, the dose of aspirin used in our studies (100 mg/kg in rats) is much higher than the 1-1000 mg/day (total dose) in humans, it is still in the therapeutic range for rats. The reason for the difference in dose from rats to humans can be due to multiple factors influenced by the absorption, metabolism and elimination of the aspirin, and is not clearly understood.

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[4] Steiner et al., *Aspirin in Episodic Tension-Type Headache: Placebo-Controlled Dose-Ranging Comparison with Paracetamol*, Cephalalgia, Feb; 23 (1): 59-66, 2003.
[5] ten Berg et al., *High-Dose Aspirin in Addition to Daily Low-Dose Aspirin Decreases Platelet Activation in Patients Before and After Percutaneous Coronary Intervention*, Thromb. Res. Mar; 105 (5): 385-90.
[6] Aguejouf et al., *Effects of Acetyl Salicylic Acid Therapy on an Experimental Thrombosis Induced by Laser Beam*, Thromb. Res. Sep 15; 99(6): 595-602, 2000.
[7] Vesvres et al., *Effects of Aspirin on Embolization in an Arterial Model of Laser-Induced Thrombus Formation*, Haemostasis. 23(1): 8-12, 1993.
[8] Killackey et al., *The Effects of High Doses of Aspirin and Related Benzoic Acid Derivatives on Arterial Thrombosis in Male Rats*, Haemostasis. 14(4): 354-60, 1984.
[9] Schumacher et al., *Superior Activity of a Thromboxane Receptor Antagonist as Compared with Aspirin in Rat Models of Arterial and Venous Thrombosis*, J Cardiovasc Pharmacol. Oct; 22(4): 526-33, 1993.
[10] Lockyer et al., *Demonstration of Flow and Platelet Dependency in a Ferric Chloride-Induced Model of Thrombosis*, Cardiovasc Pharmacol. May; 33(5): 718-25, 1999.
[11] Hirose et al., *Antiplatelet and Antithrombotic Effects of a Novel Selective Phosphodiesterase 3 Inhibitior, NSP-513, in Mice and Rats*, Japanese J Pharmacol. Mar; 82(3): 188-98, 2000.
[12] Schumacher et al., *A Ferret Model of Electrical-Induction of Arterial Thrombosis That is Sensitive to Aspirin*, J Pharmacol Toxicol Methods. Feb; 35(1): 3-10, 1996.

9. In the above study, rats were dosed with ezetimibe at 3 mg/kg/day, which was previously found to be the maximally effective dose to prevent diet-induced hypercholesterolemia in rats.[13] Therefore doses ranging from 0.1-1000 mg/day, with the usual dose of 10 mg/day, should be effective clinically in humans.

I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that those statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 1/4/2007

Madhu Chintala, Ph.D.

[13] van Heek et al., *Ezetimibe Potently Inhibits Cholesterol Absorption But Does Not Affect Acute Hepatic or Intestinal Cholesterol Synthesis in Rats*, British Journal of Pharmacology 138: 1459-1464, 2003.